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STUDY OF CHEMICAL CONTROL OPTIONS AGAINST CHESTNUT BLIGHT DISEASE

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ABSTRACT
Cryphonectria parasitica, the causal agent of chestnut blight, is a destructive *Ascomycota* fungal disease infecting European chestnut (*Castanea sativa*) and American chestnut (*Castanea dentata*) trees. On susceptible host trees, necrotic lesions (cancers) are caused by the disease on the bark of the trunk and branches. The disease leads to wilting and destruction of the chestnut trees.
Protection against the pathogen is difficult. Biological control using hypovirulent strains of the pathogen is one of the best options, but its implementation requires a lot of preliminary testing, as well as compatibility between the virulent pathogen strain that infects the area and the hypovirulent strain used for treatment. Thus, this procedure is a rather complicated and slow process. Chemical control against the pathogen is not currently widespread, as its implementation is also difficult due to the large size of the trees and the nature of the forest-like plantations, and there are currently no available pesticides. At the same time, protection with chemical pesticides may be feasible in plantations in which the size of the trees allows for application (lower-sized trees or young plantations). Therefore, it is necessary to find fungicides that can be used effectively, which is the purpose of this experiment.
In vitro efficacy of four chemical pesticides [Pictor (*dimoxystrobin+boscalid*), Amistar Sun (*azoxystrobin+difenoconazole*), Score 250 EC (*difenoconazole*), Cuproxat FW (*tribasic-cooper-sulphate*)] has been tested against *Cryphonectria parasitica*. According to the results of this experiment Score 250 EC and Amistar Sun were the most effective fungicides, given that they inhibited fungal growth even at the lowest concentrations of the test solution.

Keywords: *in vitro*, *Cryphonectria parasitica*, chemical control, mycelial growth

INTRODUCTION

Chestnut (*Castanea sativa* Mill.) are notably important in Europe. The fruits are rarely used for raw consumption, but frozen products are highly popular mainly because of their year-round availability. Besides the fruits, the bark, leaves, and flowers are used in pharmacopoeia. The honey obtained from chestnut is also highly sought-after because of its pleasant. (VASCONCELOS ET AL., 2010)

Chestnut blight caused by fungal agent *Cryphonectria parasitica* (Murr.) Barr (syn: *Endothia parasitica* [Murr.] And.) is a destructive disease-causing necrotic lesion (cankers) on the bark of the trunk and branches on susceptible chestnut trees eventually leading to wilting and complete destruction of the tree (RADÓCZ & TARCALI, 2009). Protection against the pathogen is a difficult task. Eradication efforts by collection and destruction of infected plants parts have mostly failed.
Interspecific hybrids between *Castanea sativa* and *Castanea crenata* exhibited promise able tolerance to *Cryphonectria parasitica* (CHIRA ET AL., 2018). Using resistant varieties can help control chestnut blight, but it is a time-consuming process.
Hypovirulence is a natural phenomenon which was discovered on European chestnut trees opening opportunities for biological control (ROBIN & HEINIGER, 2001). Biological control

using hypovirulence is promising and widely used against European chestnut blight. The success of biological control depends on finding the ideal environmental or biological conditions, and hypovirulence is no exception. Additionally, its implementation required a lot of preliminary testing, diversity in vegetative compatibility types of *Cryphonectria parasitica*, which makes this procedure a rather complicated and slow process (MILGROOM & CORTESI, 2004). Control of the pathogen by means of hypovirulence and resistant varieties shows potential, but these techniques are slow and long-winded.

Implementation of chemical control against chestnut blight is also limited because of the large size of the trees and forest like plantations, nonetheless fungicidal protection could be feasible in young plantations. Over the years, blight cankers have been treated with fungicides like captan, epoxiconazole, carbendazim, azoxystrobin, folpet, etc. but some of them have shown to be ineffective in the long-term process. Moreover, some effective fungicides have been withdrawn under the new EU laws which further confines the selection of fungicides accessible for exercising control against the pathogen. Thus, it is necessary to determine which fungicides can be effectively used in control against *Cryphonectria parasitica*.

MATERIALS AND METHODS

Sample collection

Samples were collected on 13 January 2022 in Rezi (Zala County, Hungary) with a sharp sampling knife from an approximately 20-year-old chestnut orchard. The collected samples were placed in a collection bag one by one, and we brought to the laboratory of Plant Protection Institute of University of Debrecen.

Preparation of pure cultures

Pure culture colonies of *Cryphonectria parasitica* were prepared by surface sterilization of the bark samples with 70% ethanol. The samples were then washed in sterile, distilled water and placed on Potato dextrose agar plates. The culture was incubated under dark conditions at 25±2°C and preserved for further tests in the laboratory of Plant Protection Institute of University of Debrecen.

In vitro testing of *Cryphonectria parasitica* using fungicides

For this study, four widely accepted fungicides viz. Pictor, Cuproxat FW, Score 250 EC and Amistar Sun and were evaluated for their efficacy on mycelial growth of an *Cryphonectria parasitica* using poisoned food technique (GROOVER AND MOORE, 1962; SHAHI ET AL.,1999, PUNDIR ET AL.,2010).

During the experiment, these fungicides were tested at four different concentrations to test the mycelial growth of the pathogen by using a poisoned media technique in vitro. Four stock solutions were prepared with different concentrations of fungicides. The final concentration at which the fungicides were tested were 10ppm, 50 ppm, 100 ppm and 500 ppm with three replicates. In the first step, 1000 µl of every stock solution was pipetted and mixed with 20 ml potato-dextrose-agar media at 60°C in 50 ml Falcon tubes.

In the second step, the liquid media was mixed with the fungicide in a falcon tube by subjecting to vortex for four seconds and the 21 ml media with fungicide was poured into 90 mm Petri-dishes. After the solidification of media, mycelial plugs (6 mm diameter) from *Cryphonectria parasitica* from 7-day-old pure culture were placed upside down into the center of petri plates with the poisoned PDA. As a control, test fungal growth was observed on PDA that had not been poisoned. The petri-dishes with the cultures were incubated under dark conditions at 25±2°C and observations on the mycelial growth of test fungus were recorded on the third and sixth day.

The percent inhibition in mycelial growth due to various fungicidal treatments at different concentrations was computed using the following formula:

$$\text{Mycelial growth inhibition (\%)} = \frac{dc-dt}{dc} \times 100(\%)$$

Where; dc = average diameter of fungal colony in non-poisoned PDA (control), and dt= average diameter of fungal colony in Poisoned PDA (NISA ET AL., 2011, SHAKEEL ET AL., 2021).

Table 1. Name and active ingredients of fungicides involved in the study

Name of the Fungicides	Ingredients
Pictor	*200 g/l dimoxystrobin + 200 g/l boscalid*
Cuproxat FW	*350 g/l tribasic-cooper-sulphate*
Score 250 EC	*250 g/l difenoconazole*
Amistar Sun	*200 g/l azoxystrobin+ 125 g/l difenoconazole*

RESULTS

Mycelial growth of *Cryphonectria parasitica* was measured on the third and sixth day. The average diameter of control mycelia was 19.90 mm and 22.50 mm on the third and sixth day respectively.

Based on the recordings of the third day Pictor exhibited slight inhibition only at 500 ppm concentration. Cuproxat FW showed complete inhibition at 500 ppm. Score 250 EC and Amistar Sun exhibited promising results as there was no fungal growth even at 10 ppm concentration.

Table 2. Inhibition of the mycelial growth in percentage on the third day

Fungicides	**10 ppm**	**50 ppm**	**100 ppm**	**500 ppm**
Pictor	-5,53	-5,53	-0,50	22,11
Cuproxat FW	-53,27	-45,73	-28,14	100,00
Score 250 EC	100,00	100,00	100,00	100,00
Amistar Sun	100,00	100,00	100,00	100,00
Control (mm)	19,90			

On the sixth day, Pictor was ineffective even at 500 ppm whereas Cuproxat FW exhibited over 50% inhibition of mycelial growth at 500 ppm. Score 250 EC and Amistar Sun maintained 100 % inhibition of fungal growth even at 10 ppm.

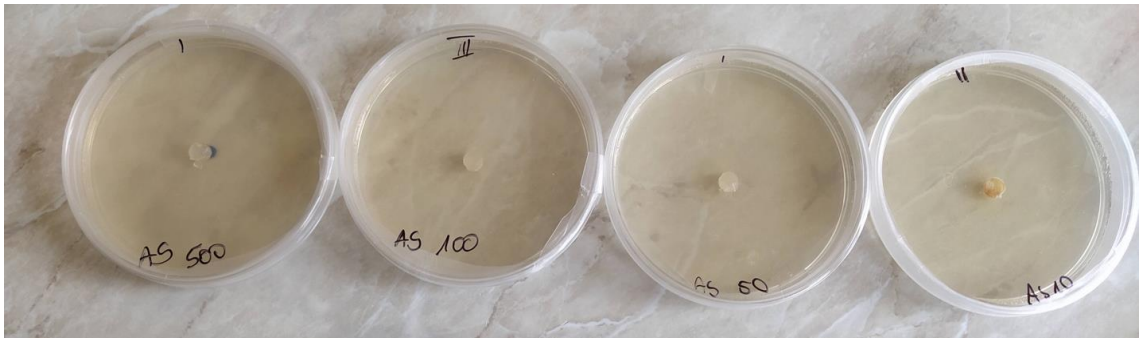
The results suggest that Score 250 EC and Amistar Sun were the most effective fungicides, given that they inhibited fungal growth even at the lowest concentrations of the test solution. They exhibited hundred percent inhibition rate of mycelial growth of *Cryphonectria parasitica* even at 10 ppm concentration which is the lowest concentration of the test solutions. Cuproxat was effective only at 500 ppm concentration. Pictor did not exhibit significant inhibition of the mycelial growth of the fungicides.

Table 3. Inhibition of the mycelial growth in percentage on the sixth day

Fungicides	**10 ppm**	**50 ppm**	**100 ppm**	**500 ppm**
Pictor	-66,67	-38,67	-39,19	-17,57
Cuproxat FW	-108,89	-111,11	-88,89	51,11
Score 250 EC	100	100	100	100
Amistar Sun	100	100	100	100
Control (mm)		22,50		

Figure 1. Mycelial growth on sixth day on PDA-media poisoned with Cuproxat FW

Figure 2. Mycelial growth on sixth day on PDA-media poisoned with Amistar Sun



DISCUSSION

The results suggest that Score 250 EC and Amistar Sun could be effective *in vitro* against *Cryphonectria parasitica.* Both preparations contain the active substance difenoconazole, so according to experimental results, this active substance can be effective against the pathogen. *In vitro* tests are quick and simple to carry out, and they can reveal whether a product is beneficial. However, several variables, including degradability, persistence, mechanism of action, and interaction with other chemicals, will affect this outcome (GONZÁLEZ-VARELA & GONZÁLEZ, 2007). However, further studies should be carried out to evaluate the *in vivo* effectiveness of the fungicides.

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